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AN EXPERIMENTAL INVESTIGATION INTO THE  
OCCURRENCE AND SIGNIFICANCE OF SUGAR IN  
NORMAL AND PATHOLOGICAL URINES.

being

A THESIS PRESENTED FOR EXAMINATION FOR THE DEGREE OF  
M. D.

by

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## I N T R O D U C T I O N .

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Urinary analysis has always been a subject of importance and has afforded scope for investigation to nearly all chemists who have been interested in clinical phenomena. The literature on the subject is so vast that it is a matter of difficulty to review all that has been done, and even more difficult to estimate the value of many of the contributions.

The methods of chemical analysis are now so exact that the question of the sugar present in normal and pathological urine might be thought to admit of little further investigation all the facts relating to it being already well known. Such, however, is far from being the case.

The presence of traces of sugar in urine has been satisfactorily proved, but there are still differences of opinion on such points as the amount actually present in normal urine, its significance, and as to what are the best methods of estimating it.

In the routine examination of urines, ambiguous reactions are frequently met with, and since some knowledge of the significance of these reactions is obviously of great importance, it is disappointing to find that a perusal of the ordinary text books on urine analysis fails to give the desired information.

In this research an attempt has been made to

ascertain the average quantity of sugar present in normal urine by methods differing in many respects from those already employed. The cause and significance of many ambiguous reactions have also been investigated.

As a result of a considerable number of experiments it would appear that sugar, in amount somewhat beyond the normal, is present much more frequently than is generally supposed, and that in many cases in which the ordinary tests give modified results - wrongly ascribed to "interfering" substances - the cause is really due to the presence of sugar in slight excess.

The readiness with which sugar appears in the urine of many individuals as the result of various factors is at present hardly recognised by the medical profession, and for this, the unsatisfactory treatment of the subject by the text books is largely to blame.

The question is of great importance from the point of view of Life Insurance, for it has been repeatedly found that the urine of different applicants for insurance gives a slight sugar reaction as the result of indulgence - sometimes little - in alcoholic beverages. In these cases the removal of the cause always results in the disappearance of the sugar.

From the clinical standpoint the subject is one



which demands much further investigation for the significance of a constant occurrence of slight excess of sugar in the urine can be ascertained only by watching the cases for a prolonged period of time.

This investigation has been carried out in the Pathological Chemistry Laboratory of St. Thomas's Hospital, and for permission to work there I am indebted to the committee of management. My thanks are also particularly due to the Director, Dr. Maclean, for the facilities he has so generously placed at my disposal.

## CHAPTER 1.

### THE PRESENCE OF SUGAR IN NORMAL URINE.

Human blood contains between 0.1 and 0.2 per cent of glucose so one would expect to find some sugar present in normal urine. For many years, however, normal urine was considered to be free from sugar and the statement of Brücke (1) in 1858 that all urines contained a trace of sugar was the subject of much controversy and criticism.

The complex nature of the urinary secretion renders it very difficult to identify sugar by any direct chemical means, because urine contains so many different substances, some of which react more or less like sugar to one or other of the sugar tests. More important even than this is the fact that certain substances present, obscure the normal reaction of sugar. Thus, sugar can be identified by Fehling's method when present in a solution of only 0.008 % when distilled water is used as the solvent, but the addition of much more than this amount to normal urine gives no reaction. This at once proves that urine contains something which prevents sugar, when present in small amount, from giving any appreciable reaction with Fehling's solution and explains why normal urine, when tested in the usual way, appears to be sugar free. These objections, however, are not applicable to

certain colour tests. Molisch (2) and Luther (3) using alpha-naphthol with thymol and furfur-aldehyde obtained positive results. These results were corroborated by Wedenske (4) and Baisch (5) who treated the urine with benzoylchloride and in this way precipitated any glucose that might be present in the form of the ester. On treating the ester with sodium hydrate sugar was identified. Positive results were obtained with phenyl-hydrazine by Allen (6) and Breul (7). Pavy (8) made use of lead oxide and obtained a substance which had all the ordinary reactions of glucose. Lohnstein (9) applied the fermentation test to urine in many cases with positive results, and Maclean (10) and others using Safranin obtained undoubted evidence of the presence of sugar.

Owing to the complex conditions under which the sugar must be identified many observers denied its occurrence in normal urine. Among these may be mentioned Maly (11), Kulz (12), Friedlander (13) and Johnsons (14). These observers ascribed the reactions due to its presence, to Creatinin, Glycuronic Acid and other substances.

A general review of the literature leaves no doubt that the presence of sugar in small amount in normal urine has been proved, but the actual amount present is by no means agreed upon. Thus Lohnstein gives the figure at 0.001 %, while Pavy stated .1 %



might be taken as an average.

A consideration of the different tests used helps to clear up many of the anomalies found in the literature. Of these tests one of the most frequently employed is that of Fehling, and a short survey of the effect of certain urinary constituents on its reaction with sugar may help to explain these anomalies.

#### FEHLING'S SOLUTION.

When normal urine is treated with Fehling's solution no reaction is obtained, although for the detection of sugar the reagent is one of the most delicate at our disposal. Maclean (1) has shown this to be due to the fact that the Creatinine present in urine has the property of holding any reduced cuprous oxide in solution, so that although the sugar present reduces its equivalent amount of copper, no evidence of the reaction is obtained.

The cuprous oxide, which in the case of an aqueous solution would be at once precipitated, is held in solution by the Creatinine. If, however, there is slight excess of sugar and the cuprous oxide formed is greater in amount than can be held in solution by the Creatinine present, a result is obtained which is often met with in urinary analysis. No precipitate comes down on heating in the usual way but after a shorter or longer interval the reduced oxide begins to separate in an exceedingly finely divided, and more or

less colloidal form which gives to the solution a muddy green appearance; such a mixture it is quite impossible to filter.

If rather more sugar is present the effect of the Creatinine is less in evidence and a greenish looking precipitate is obtained which may be filtered with difficulty. According to the amount of sugar present precipitates varying in colour from yellow to bright red are obtained. From this it is clear that no difficulty arises either in the qualitative or quantitative testing for sugar, where comparatively large amounts are present. On the other hand the presence of small amounts leads to ambiguous and often misleading results.

In all these cases the creatinine present reduces a certain amount of Fehling's solution, but as creatinine is only destroyed slowly by the alkali of Fehling's solution, the actual amount of the reagent reduced depends on the time during which the urine and the Fehling's solution are boiled. From this it is evident that any slight reduction given by a urine after prolonged boiling is no indication of the amount of sugar present, for the longer the boiling is continued the greater is the amount of reduction.

It may be mentioned here that all urines give a well marked reduction with Fehling's solution if boiled long enough: this reduction generally begins to



appear after 3 to 4 minutes boiling.

In all experiments in which attempts are made to estimate the total reducing power of urine on Fehling's solution and <sup>on</sup>/solutions such as Pavy's and Bertrand's, no significance can be attached to the figures unless the time during which the mixtures are boiled is given.

Unless the time is known all these experiments are useless, for a urine which gives a certain reduction when boiled for 3 minutes may give twice or three times as much when boiled for 4 or 5 minutes.

On that account the experiments of Funk (1) which are quoted as the latest results on the question of the amount of sugar in normal urine are of no value.

Funk boiled mixtures of urine and alkali  $\text{Cu SO}_4$  for certain periods and obtained some reduction. He endeavoured to separate the reduced suboxide from the alkali solution by filtering through asbestos. In many cases only part of the precipitate was collected, part being so finely divided that it passed through the filter. His calculations are based on the erroneous supposition that the retained oxide corresponds to the sugar present, while the finely divided part corresponds to the other reducing substances such as Uric Acid, Creatinine, Creatine &c.

That a few of his results agree with the figures

obtained by other observers is accidental.

They vary from 0.002 % to .04 % glucose.

The total reducing power of urine is, in the light of the above statements, of little importance but a general idea of the figures found is indicated by the following table:-

Observer.	Reagent solution	Reduction calculated as percentage of glucose.
Flückiger (1)	Fehling's sol:	0.15 % - 0.25 %
Salkowski (2)	" "	0.25 % - 0.60 %
Moritz (3)	Pavy's "	0.11 % - 0.36 %
Gregor (4)	" "	0.08 % - 0.35 %
Schoendorff (5)		0.011% - 0.027% Sometimes .1 %
Lavesson (6)	Bang's sol:	0.238 %
Funk (7)	Bertrand's sol:	0.002% - 0.04 %

(1) Zeits für physiol. Chemic 9, 323 (1885).

(2) " " " " 17, 229 (1893).

(3) Archiv für Klin Medizin 46, 217 (1890).

(4) Centralb. für Krankh. d. Harn-u Sexualorg.  
10, 240 (1899).

(5) Archiv f. d. ges. Physiol. 121, 572. (1908).

(6) Biochem, Zeits. 4, 40 (1907).

(7) Zeits für physiol, Chemie. 69, 72 (1910).

Owing to the presence of the other reducing substances these figures are of themselves of no value as indicating the actual amount of sugar present in urine. Estimation of the reducing power before and

after fermentation with yeast should give a fairly definite answer to the question and many experiments have been carried out with this object in view. It has been pointed out, however, that in yeast itself certain reducing bodies - gummy matters, purines &c., are formed during incubation, so that experiments in which this fact has been ignored lose greatly in value.

A series of experiments was carried out with yeast which had been thoroughly washed two or three times by means of the centrifuge, and it was found that such yeast gave little or no reduction of itself when incubated for several hours at 37°C. A full account of these experiments is given later.

In another series the "interfering" substances were precipitated with mercuric chloride, and the reducing power of the filtrate estimated. The amount of fermentable substance in another portion of the filtrate was determined at the same time.

Besides these an entirely new method was adopted depending on the great ease with which caustic alkalies destroy sugar.

The whole of the sugar in a .1 to .2 % solution is destroyed when the solution is boiled for about a minute with the alkali part of Fehling's solution. The other constituents of the urine are not appreciably changed in that time so that the difference in reducing power of two specimens, one of which has been



treated with alkali, gives a fair indication of the amount of sugar present.

This observation is of importance from a practical point of view, for in any urine in which a slight Fehling's reaction is obtained, such reaction cannot be due to sugar if it persists after preliminary boiling with alkali.

A comparison of the results obtained by these different methods showed that they agreed within fairly narrow limits, and a series of experiments with Safranin gave closely similar figures.

Unlike many reagents used in testing for sugar safranin is unaffected by uric acid, hippuric acid, creatine, creatinine, chloral, chloroform, pyrocatechin or hydroquinone. According to Maclean (1) it reacts with all the ordinary carbohydrates except perhaps starch and raffinose. With the polysaccharides (Dextrin and Glycogen) the reaction is not very marked and is probably dependent to some extent on the presence of impurities. With cane sugar, lactose and maltose, as well as with the monosaccharides, galactose and laevulose, glucose it gives a well marked reaction.

Of the pentoses, xylose and arabinose give positive results, and glycuronic acid gives a marked reaction.

The test depends on the fact that carbohydrates in the presence of an alkali discharge the red colour of the safranin solution, the liquid changing to a pale straw-yellow colour. On shaking the fluid,

oxidation takes place and the red colour reappears.

None of the ordinary constituents of urine interfere with this test so it is an excellent one for determining the presence of carbohydrate.

The results obtained - (.07% to .11% carbohydrate) - when estimated as glucose appear to be too high for normal urine, but this may be because it is probable that all the carbohydrate present in urine is not glucose, as would appear from the results obtained by hydrolysing urine with weak acids.

#### A.

Reduction experiments before and after boiling the urine with sodium hydroxide.

In these experiments Bertrand's solution was used, which has the following composition:-

No. 1. (Copper Solution).

Copper sulphate 40 gm.  
Distilled water to 1000 c.c.

No. 2. (alkali solution).

Sodium Potassium Tartrate 200 gm.  
Sodium Hydroxide. 150 gm.  
Distilled water. 1 litre.

At the beginning of this investigation experiments were made to determine the time during which boiling had to be continued in order to destroy all the sugar, when a .1% sugar solution was mixed with half its volume of the alkali part of Bertrand's solution.

It was found that this was accomplished in about 30 seconds.

After boiling with the alkali the copper sulphate solution was added, the whole boiled for 3 minutes, and the sugar estimated according to the directions given by Bertrand.

1. Bullet de la Societe Clinique 35 1285 (1906).

Experiment 1.

Amount of .1% sugar solution used.	Time boiled in seconds.	Amount of alkali (Bertrand's sol:)	Result.
20 c.c.	10	10 c.c.	Some sugar still present.
20 c.c.	15	10 c.c.	" "
20 c.c.	20	10 c.c.	Trace.
30 c.c.	30	10 c.c.	No sugar left.

From the above it would appear that the sugar in a .1% solution is destroyed when it is boiled for 30 seconds with half its volume of Bertrand's alkaline solution. This solution contains 150 grm in 1000 c.c., so the amount in 10 c.c. is ~~about~~ 1.5 grm.

Another experiment was carried out in which 20 c.c. of a similar sugar solution was boiled for 30 seconds with different amounts of alkali.

Experiment 2.

	Result.
1. 20 c.c .1% glucose with 5 c.c 16% NaOH for 30 secs.	Still contains sugar.
2. 20 c.c .1% glucose with 7 c.c " " "	"



3. 20 c.c. 1% glucose with 8 c.c 16% NaOH for 30 seconds. Result: Trace.
4. 20 c.c. 1% glucose with 10 c.c 16% NaOH for 30 seconds. Result: No sugar left.

These results prove that a concentration of 1%, sugar is completely destroyed when boiled for 30 seconds with half its volume of an alkaline solution, 1.c.c. of which contains 0.15 to 0.16 gram., - or a 15-16 % solution.

Similar results are obtained by using Pavy's method of estimating sugar.

The next point was to determine the effect on the other constituents of the urine of boiling for a similar period with the same strength of alkali. The substances investigated were Uric Acid, Creatinine, Creatin and Glycuronic Acid.

As certain manipulations were rather difficult when carried out by Bertrand's method, recourse was had to the use of Pavy's method in all these cases except the last.

The Pavy's solution used was such that 10 c.c. corresponded to .0047 gm glucose, and the method adopted was as follows:-

The reagent diluted with three times the amount of distilled water was heated to boiling, and the bulk of the test solution necessary for reduction added during the first minute. Boiling was continued for 2 more minutes, and the amount of the

test solution necessary to complete the reduction at the end of the time noted.

# 1. Uric Acid.

A .15 % solution of Uric Acid in dilute alkali was tested as above with 5 c.c. of Pavy's solution, and the following results obtained:-

Burette readings.

13.45 c.c.	16.50	19.60	
10.40	13.45	16.50	
<hr/>	<hr/>	<hr/>	
3.05	3.05	3.1	Average 3.07 c.c.
<hr/>	<hr/>	<hr/>	

∴ .0092 grm uric acid = .0047 grm glucose.

20 c.c. .3 % solution of uric acid in dilute alkali was boiled for 30 seconds with 1.5 gram NaOH, nearly neutralised and diluted to .15 %, and treated as above.

Burette readings.

23.3	26.7	30	
19.8	23.3	26.7	
<hr/>	<hr/>	<hr/>	
3.5	3.4	3.3	Average 3.4
<hr/>	<hr/>	<hr/>	

.01 grm = .0047 grm glucose.

2. Creatine. .15 % solution treated in the same way.

Burette.

44.9	31.8	42.8	24.35	37.1
33.8	30.7	31.8	13.3	26.1
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
11.1	11.1	11.0	11.05	11.0
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
Average 11.05.				

.03315 grm creatine = .0047 grm glucose

After treatment with alkali.

28.85	39.8
18.00	28.85
<hr/>	
10.85	10.95
<hr/>	

$$.0327 \text{ gm} = .0047 \text{ gm glucose.}$$

### 3. Creatinine.

Solution prepared by hydrolising .3 gram creatin with 20 c.c. 5%  $\text{HCl}$ . Solution then neutralised and made up to 100 c.c with distilled water.

A .15 % solution was treated as above.

Burette.

24.0	28.4	32.8	37.1	
19.35	24.0	28.4	32.8	
<hr/>				
4.65	4.4	4.4	4.3	Average 4.44
<hr/>				

$$.01332 \text{ gm} = .0047 \text{ gm glucose.}$$

After treatment with alkali.

29.15	34.70	40.20	45.8	
23.65	29.15	34.70	40.2	
<hr/>				
5.5	5.55	5.50	5.6	Average 5.54.
<hr/>				

$$.01732 \text{ gm} = .0047 \text{ gm glucose.}$$

4. Glycuronic Acid obtained by hydrolysing "Indian Yellow". With Pavy's solution it was found very difficult to get an exact end reaction so the estimation was carried out by Bertrand's method as follows:-



20 c.c. of the solution of glycuronic acid was diluted with an equal quantity of distilled water.

20 c.c. of this diluted solution was boiled for 3 minutes with 20 c.c. of each Nos. 1 and 2, Bertrand's solutions.

The liquid was then filtered through asbestos under pressure and the precipitate of cuprous oxide washed with distilled water. The washed precipitate was dissolved in Bertrand's solution No 3. (Ferric sulphate) and the resulting solution titrated with Potassium Permanganate (1 c.c  $K_2Mn_2O_8$  = 10.1 mgr copper) This experiment was repeated twice and the permanganate used on each occasion amounted to 4.0, and 4.05 c.c

40.64 mgr Cu = 10.3 mgr glucose.

20 c.c. of the glycuronic acid solution was then treated with alkali and also estimated by Bertrand, with the result that only .1 c.c. of permanganate solution was used.

These experiments show that the reducing power of Uric Acid and Creatine is practically unaffected by boiling for 30 seconds with alkali, that of Creatinine is only slightly, while that of glycuronic acid is completely destroyed.

Since normal urine contains only traces of glycuronic acid its destruction by alkali is of little importance, and a consideration of the whole results show that a deduction of about 10% for the action of the alkali on these urinary constituents is ample

allowance. The remaining difference between the amount of reduction before and after boiling with alkali may be ascribed to sugar.

A series of urines was tested with Pavy's solution before and after boiling with alkali. In the test the urine was diluted with an equal quantity of distilled water, and 10 c.c. Pavy's solution used diluted to 40 c.c.

The alkali was sodium hydrate and in using it 20 c.c. of urine was boiled with 1.5 gram NaOH for 30 seconds, neutralised with hydrochloric acid and made up to 40 c.c., so as to compare exactly with the untreated urine.

The reaction and specific gravity of each urine were taken and a note made of any change obtained by boiling with an equal quantity of Fehling's solution for 30 seconds.

An examination of the table shows that in 15 cases the average amount of urine - diluted 1 in 2 - required to reduce 10 c.c. Pavy's solution was 6.962 c.c. Allowing for dilution the average amounts to 3.481 c.c urine = 10 c.c. Pavy.

After boiling with alkali the average amount of urine required to decolorise 10 c.c. Pavy, allowance being made for dilution, was 5.582 c.c.

10 c.c. Pavy's solution = .0047 gram glucose.  
therefore 3.481 c.c. urine = .0047 gram glucose  
= 1.35 gram per 1000 c.c. urine.

after treatment with alkali

5.582 c.c. urine = .803 gram per 1000 c.c. urine.

Difference

1.35 gram - .803 gram = .547 gram per 1000 c.c. or  
.0547 %

If allowance of 10 % be made for action of alkali on other reducing substances such as creatinine and glycuronic acid, we get an average of about 0.05 % for 15 urines, with variations from .036 % to .064 %



The figures given in the table each represent the average of three experiments.

Urine (20 c.c. diluted to 40 c.c.) Pavy 10 c.c. = .0047 grm glucose.

	<u>1.</u>	<u>2.</u>	<u>3.</u>	<u>4.</u>	<u>5.</u>	<u>6.</u>	<u>7.</u>
Sp: Grav: Reaction. Fehling 30 secs:	1014 Acid. Nil.	1016 Acid. Nil.	1022 Acid. Slight Decolor:	1012 Acid. Nil.	1017 Acid. Decolor:	1013 Neutral. Slight Decolor:	1016 Acid. Nil.
Amount of un- treated urine used. <i>Grav. 10 c.c. Pavy.</i> Amount used after treatment with alkali.	7.6 14.36	7.183 13.583	4.733 6.633	9.75 16.0	7.133 11.4	8.733 13.283	7.766 12.133
	<u>8.</u>	<u>9.</u>	<u>10.</u>	<u>11.</u>	<u>12.</u>	<u>13.</u>	<u>14.</u> <u>15.</u>
Sp: Grav: Reaction. Fehling 30 secs:	1015 Acid. Nil.	1018 Acid. Greenish	1018 Acid. Green.	1010 Acid. Nil.	1027.5 Acid. Slight decolor:	1020 Acid. Slight Decolor:	1019 Acid. Slight decolor: 1016 Acid. Slight decolor:
Amount of un- treated urine used. Amount used after treatment with alkali.	8.483 14.600	6.05 9.83	6.166 10.3	8.333 17.15	4.633 5.91	5.466 8.666	6.566 11.766 6.266 9.933



B.

THE CHANGE IN THE REDUCING POWER OF NORMAL URINE AS  
THE RESULT OF FERMENTATION WITH YEAST.

Several experiments were made, with safranin as an indicator of the change.

Urines were incubated with washed yeast for 12 to 18 hours in the presence of Toluol.

The yeast was then filtered off and the reduction estimated by ascertaining the number of drops of a '1 c.c. safranin solution which 5 c.c. of the fluid was capable of decolorising in the presence of 1 c.c. 10% sodium hydroxide.

Heating was carried out in a beaker of boiling water, and comparisons made with known concentrations of sugar.

In six cases investigated the following results were obtained. The figures given are in terms of glucose.

Sp: Grav:	Before yeast, carbohydrate present.	After yeast	Difference fermentable sugars.
1. 1020	0.13 %	0.06 %	0.07 %
2. 1016	0.10 %	0.04 %	0.06 %
3. 1015	0.12 %	0.06 %	0.06 %
4. 1021	0.08 %	0.03 %	0.05 %
5. 1018	0.07 %	0.02 %	0.05 %
6. 1015	0.08 %	0.03 %	0.05 %
		Average	0.0567 %



In a few cases Bertrand's method was used for estimating the reduction but as the filtration of the precipitate is impossible if the experiment is carried out in the ordinary manner and the solutions boiled for only 3 minutes, the solution was boiled for 10 minutes using a reflux condenser, and then 3 minutes without a condenser.

A.			
Sp: Grav:	1012	1017	1015
Reaction.	Neutral.	Acid.	Acid.
Fehling's test <i>when applied in the ordinary clinical way gave.</i>	Green Colour.	Green colour.	Nil.
Permanganate. in Bertrand's method.	3.9 c.c. = 19.6 mg. glucose.	5.35 = 27.5 glucose.	4.95 = 25.5 glucose.

B. 30 c.c. of each specimen was incubated with one gram of washed yeast for 40 hours in the presence of Toluol. Controls of 30 c.c. .2% glucose and 30 c.c. distilled water each with one gram of yeast.

The yeast was filtered off and the urine treated as above.

	<u>1.</u>	<u>2.</u>	<u>3.</u>	Controls	
				Glucose	
Permanganate. used.	1 c.c.	2.95 c.c.	2.95	.2 c.c.	.1
	4.9 m.g. glucose.	14.8	14.8	.98	.49

Percentage of reducing substances calculated as sugar.

A. Before fermentation	.098	gram	.1375	gram	.1275	gram
B. After	"		.0245	"	.0740	"
Sugar fermented by yeast in %			.0735		.0635	.0535

Average = .0635 % Glucose.



C.

EXPERIMENTS WITH MERCURIC CHLORIDE AND YEAST.

As already mentioned it was found impossible to estimate the reducing power of normal urine by Bertrand's method as carried out in the ordinary way. This is due to the fact that the reduced cuprous oxide is present in colloidal form and cannot be filtered. This difficulty can, in certain cases, be overcome by boiling the urine for a period longer than that recommended by Bertrand. Bertrand's tables are worked out for sugar solutions which have been boiled for 3 minutes, and as an extension of the time of boiling gives somewhat different results. An attempt was made to find some means by which the method could be applied in strict accordance with Bertrand's directions.

The difficulty of filtration of the cuprous oxide obtained after boiling for a short time depends on the presence of the urinary creatinine. When a weak solution of sugar and creatinine is boiled with Bertrand's solution a very finely divided precipitate is formed, almost all of which will, in many cases, pass through the finest filter paper. If the mixture is treated before-hand with mercuric chloride to precipitate the creatinine, and the mercury subsequently removed by hydrogen sulphide, the liquid obtained

gives, on boiling with Bertrand's solution, a well marked granular precipitate which presents no difficulty on filtration. In the following experiments urines were treated with mercuric chloride and allowed to stand for 24 hours. Along with creatinine other interfering substances such as uric acid were precipitated. On separation of the mercury it was found that Bertrand's method could be subsequently applied to estimate the total reduction.

The urine which had been treated with mercuric chloride was then subjected to the action of yeast to ascertain how much of the substances not precipitated was fermentable. It was found that mercuric chloride precipitated nearly all the unfermentable material as shown by the following results:-

Six urines were taken and to 100 c.c. of each 10 c.c. of solution of mercuric chloride was added and allowed to stand over night.

The solution was filtered; hydrogen sulphide passed through the filtrate for 15 minutes to precipitate excess of mercury, and again filtered.

Air was then bubbled through to remove the hydrogen sulphide, and 20 c.c. (=to 18.18 c.c. urine) tested by Bertrand's method, while 30 c.c. was incubated for 24 hours with 1 gram of carefully washed yeast, and 20 c.c. of this tested by Bertrand.

A control of 30 c.c. distilled water with one gram yeast was used.

Any difference in the reducing power should be chiefly due to sugar.



# EXPERIMENTS.

	<u>1.</u>	<u>2.</u>	<u>3.</u>	<u>4.</u>	<u>5.</u>	<u>6.</u>
sp: gr:	1019	1019	1009	1008.5	1019	1019
Reaction	Acid	Acid	Neutral	Acid	Acid	Acid
Result of Fehling's	Gr.	Gr.			Gr.	Gr.
qualita- tive test.	discol:	discol:	Nil	Nil.	discol:	discol:

Before yeast. Urine previously treated with  $\text{HgCl}_2$ .

Bertrand's method.

A.	<u>1.</u>	<u>2.</u>	<u>3.</u>	<u>4.</u>	<u>5.</u>	<u>6.</u>
No: of c.c. of permang: used.	1.05	5.0	1.0	1.25	4.25	5.3
Sugar present	5.9 mgr. glucose	25.5 mgr	4.9 mgr	6.18 mgr	21.46 mgr	27.06 mgr

After yeast.

Bertrand.

B.	<u>1.</u>	<u>2.</u>	<u>3.</u>	<u>4.</u>	<u>5.</u>	<u>6.</u>
Permang: used in c.c.	0.2	0.5	0.15	0.15	0.4	0.25
Sugar present	.99 mgr. glucose	2.15 mgr.	.74 mgr.	.74 mgr.	1.98 mgr.	1.24 mgr.

Control = 0.1 c.c. Permang:

Percentages of Glucose.

A.	.0325	.14	.027	.034	.118	.148
B.	.0054	.012	.004	.004	.0108	.0013
	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>
	.0271	.128	.023	.03	.1072	.1467
	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>

Average = .077 % Glucose.

average

The figures for the/percentage of sugar in normal urine obtained by the different methods are respectively:-

- 0547 % Boiling with alkali (Pavy's method of estimation).
- 0560 % Fermentation with yeast. (Safranin method of estimation).
- 0635 % Fermentation with yeast. (Bertrand's method of estimation).
- 0770 % Precipitation by mercuric chloride (Bertrand's method of estimation).  
with Fermentation with yeast.

The average of these figures is ·062 % and while ·077 % is probably a little too high, the lowest and highest numbers differ from the average to the extent of only ·01 %.

The minute percentage of sugar present in urine together with the great complexity of the liquid renders the estimation of normal glucose very difficult. It is quite probable that the fermentable carbohydrate present may not be wholly composed of glucose - and there is some evidence that it is not - but as our present day knowledge tells us nothing of the nature of the other fermentable substances it is customary to rely on the fermentation test as a true indicator of glucose. Whether or not fermentation is subject to fallacies as the ordinary reduction tests cannot be answered. It is generally accepted that any change

brought about in urine as the result of yeast action is dependent on the presence of glucose and for clinical purposes, this is deemed sufficient. The general agreement between the results obtained by NaOH and yeast is very significant, but NaOH would of course destroy other carbohydrates as well as glucose.

While it is acknowledged that glucose is the chief sugar excreted in Diabetes several investigators state that fairly large amounts of other sugars are passed as well.

The evidence put forward shows that normal urine contains .05 to .06 % of fermentable carbohydrate and the sugar is expressed as glucose.

Individual specimens are met with which contain less than this and no importance can be attached to the figures obtained unless the specific gravity of the urine is known.

In a general way the defects of Fehling's solution are a strong testimony in its favour as a clinical test from the fact that the creatinine of the urine renders it ineffective in the presence of small amounts of sugar and so makes it easy to say when a urine contains no excess of sugar. The significance of slight excess of sugar is dealt with in the following chapter.



## CHAPTER 2.

### ON THE PRESENCE OF SLIGHT EXCESS OF SUGAR IN OTHERWISE NORMAL URINES.

In the preceding chapter the difficulties are discussed which are met with in efforts made to find out by simple and direct means the amount of sugar present in normal urine.

The results as already stated indicate that .05% may be taken as a fair average. A urine containing this amount of sugar gives no reaction with the ordinary Fehling's test, but if for any reason there is a diminution in the amount of creatinine, or an excess of other reducing substances such as uric acid a more or less well marked reaction may be obtained.

It often happens, however, that the reaction is not evident until the mixture of urine and Fehling's solution has been allowed to stand for some time after boiling, and even then a greenish opalescence but no distinct precipitate is obtained.

The interpretation of such ambiguous reactions is very difficult. Do they indicate the presence of sugar in amount slightly more than normal or are they simply due to "interfering" substances?

Clinicians in general do not regard them as indications of sugar.

This point was examined in the case of certain

urines which gave ambiguous reactions with Fehling's solution, and it was found in some instances that a slight excess of sugar was present.

The methods used were those already set forth, and a repetition of the description will, therefore, not be necessary. As a result it was found that from 50 to 60% of such urines contained sugar varying in amount from .1 % or a little less to .25 %. In the case of the latter, Fehling's solution usually gave a yellowish green opalescence on boiling, with the formation of a precipitate after some time, while with the urines containing .1 % to .15 % a more or less ambiguous reaction was obtained only on standing.

From the reaction itself it was impossible to say that excess of sugar was present, but by the application of the methods already described it was shown that in many instances after fermentation with yeast the reduction both of safranin and of Fehling's solution was much diminished. Again, when such urines were boiled with alkali for a few seconds the reducing power was correspondingly diminished.

These results indicated the presence of sugar in slight excess, but the significance of such excess, whether permanent, or temporary, as the result of special factors, is difficult to estimate.

Clinically the subject is one of very great

interest and importance for we are faced with the difficulty of interpreting the significance of the presence of small, but abnormal, amounts of sugar in the urine. Our present knowledge is not sufficient to be of much real help and expert opinion varies.

Macleod (1), in his new book on Diabetes, sums up the matter as follows:-

"If there really is an excess of dextrose, however small, it indicates that something is amiss with the utilisation of carbohydrates in the organism; it is a danger signal which, if heeded, and the proper treatment applied, may unquestionably enable us to stave off the incidence of what might afterwards prove a deadly diabetes"., and Cole (2), in a recent article, emphasises the importance of being able to determine any excess of glucose, however small, above the normal.

These traces of sugar give rise to great difficulty in the case of applicants for Life Insurance, and if they indicate in general a defect in the normal carbohydrate metabolism, and are to be regarded as danger signals of an impending diabetes, their gravity is obvious.

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(1) Macleod. Diabetes: its pathological physiology  
1913.

(2) Cole: Lancet, September 20th 1913.



It may be stated, however, that such a *grave condition* is not necessarily *indicated* despite the statement of Macleod, for in almost all cases examined, in which small abnormal amounts of sugar appeared in the urine, there was no sign of the onset of diabetes. It is, of course, necessary to watch the patient for a long time and this may prove very difficult, but in four cases investigated by Maclean from five to six years ago and since examined from time to time, the urine occasionally contains slight excess of sugar, but the individuals are all quite healthy.

In all of them sugar can be induced by certain causes, one of the most important being the taking of a small amount of alcohol.

A study of the subject would make it appear that many people are exceedingly susceptible in this direction, and the presence of sugar in the urine of alcoholics is quite common.

The extreme ease with which a small amount of alcohol will produce sugar in the urine is remarkable, and it might safely be asserted that quite 50 % of normal individuals, after "dining out", pass urine with certain excess of sugar.

Excitement is also a factor in producing similar results and Folin (1), on examining the urines of certain students immediately after the ordeal of

sitting for an examination found that all of them contained sugar. The effect of drugs is well known and chloroform often gives rise to glycosuria.

Bad ventilation, so often met with in a crowded meeting, has similar effects.

Of all these the most potent factor is perhaps alcohol, but the ingestion of excess of carbohydrates must not be forgotten.

In hospital patients most of those conditions can be eliminated except those dependent on the mental state.

An investigation of the sugar tolerance of such individuals is of interest, but little has been done in this direction.

In the majority of cases examined it would appear that no decrease in tolerance was in evidence except in distinct cases of alimentary glycosuria. It may be that the cause of the condition is to be found not in a fundamental defect in carbohydrate metabolism, but is due to some circumstance whereby the glycogen present in the liver is broken down too quickly.

This raises the percentage in the blood and the kidney sets to work to eliminate the superfluous amount.

In general an examination of each individual case will often result in the cause being detected.

A careful examination of the urine must be

made, and the actual amount of sugar passed in 24 hours determined. A reaction in a specimen taken at random may simply depend on increased specific gravity and would then be of little importance.

The sugar will often disappear when the cause is removed and in such cases the appearance of sugar is of no significance as far as diabetes is concerned.

It is possible that the absorption from the intestine of certain products of bacterial action may stimulate the liver cells and produce increased breaking down of glycogen.

A few cases may represent the onset of Diabetes but the number is so small that it would be unwarrantable to make a general statement to the effect that slight excess of sugar in the urine indicates this disease. In most cases it does not do so.

In conclusion it may be stated that the ease with which glycosuria is produced in normal individuals as the result of mental or physiological stimuli such as alcohol, bad ventilation, and mental strain and excitement, is not sufficiently recognised.

In the case of applicants for Insurance the presence of occasional traces of sugar is probably, in many cases, the result of mental excitement, and should of itself be regarded as of little significance.

Frequent examination of the urine should be made



in such cases and it will often be found that the sugar soon disappears.

But great care must be exercised when the condition persists, and all known causes have been eliminated. The persistence of the reaction must then be interpreted in the light of the general condition of the patient. It is the writer's belief, however, that little importance is to be attached to the presence of small but abnormal amounts of sugar in urine.

#### CONCLUSIONS.

1. Average normal urine contains about .05 % of a fermentable carbohydrate, this carbohydrate being calculated as glucose.
2. In estimating the urinary sugar advantage may be taken of the extreme rapidity with which alkali destroys sugar.

The other reducing constituents of urine are not appreciably affected.

3. Many of the ambiguous reactions obtained in testing urine with Fehling's solution are really due to excess of sugar and not to "interfering" substances.
4. Slight excess of sugar in the urine of healthy individuals is often found to result from such

causes as mental excitement, alcohol, immoderate eating, bad ventilation &c.

5. In most cases the removal of such causes, results in a urine free from sugar in the pathological sense.
6. The evidence at present at our disposal points to the probability that in the great majority of cases slight excess of sugar is of little importance, and has no relation to Diabetes.

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